British Journal of Pharmacology (2003) 140, 159–169
www.nature.com/bjp

Ionic basis for the regulation of spontaneous excitation in detrusor smooth muscle cells of the guinea-pig urinary bladder

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1 The regulatory mechanisms of spontaneous excitation in detrusor smooth muscles of the guinea-pig urinary bladder were investigated using intracellular microelectrode and muscle tension recording techniques.

2 Detrusor smooth muscle cells exhibited nifedipine-sensitive spontaneous action potentials. Their frequency was highly sensitive to membrane polarization and was reduced by lowering the temperature. Lowering the temperature also reduced the frequency of spontaneous contractions and increased their amplitude.

3 Charybdotoxin (50 nM) and iberiotoxin (0.1 μM) increased the amplitude and duration of action potentials, and abolished after hyperpolarizations (AHPs). Both agents also increased the amplitude and duration of spontaneous contractions, and reduced their frequency. Apamin (0.1 μM) did not change the shape of action potentials but often converted individual action potentials into bursts. It also increased the amplitude and duration of spontaneous contractions, and reduced their frequency. 4-aminopyrideine (4-AP, 1 mM) increased the frequency of action potentials without affecting their shape, and increased the amplitude and frequency of spontaneous contractions.

4 Cyclopiazonic acid (CPA, 10 μM) and ryanodine (50 μM) increased the amplitude of action potentials, and suppressed AHPs. Both agents also increased the amplitude and duration of spontaneous contractions, and reduced their frequency. 1,2-(*Bis* (2-aminophenoxy) ethane-*N*,*N*,*N*′,*N*′-tetraacetic acid tetrakis (acetoxymethyl ester) (50 μM) dramatically increased the amplitude and duration of the action potential, and abolished AHPs.

5 Spontaneous action potentials in detrusor smooth muscles cells result from the opening of L-type Ca^{2+} channels, and their frequency is regulated by voltage-dependent mechanisms and by some metabolic process. Both the activation of large conductance Ca^{2+}-activated K^+ (BK) channels and Ca^{2+}-mediated inactivation of the Ca^{2+} channels are involved in the repolarizing phase of action potentials. The Ca^{2+} influx through L-type Ca^{2+} channels triggers calcium-induced calcium release *via* ryanodine receptors and activates BK channels to generate AHPs. Both small conductance Ca^{2+}-activated K^+ channels and voltage-sensitive K^+ channels may contribute to the resting membrane potential and regulate the frequency of action potentials. The regulatory mechanisms of action potentials are closely related to the regulation of spontaneous contractions.
British Journal of Pharmacology (2003) **140,** 159–169. doi:10.1038/sj.bjp.0705320

Keywords: Action potential; Ca^{2+}-activated K^+ channel and intracellular Ca^{2+} store

Abbreviations: AHP, after-hyperpolarization; 4-AP, 4-aminopyrideine; BAPTA-AM, 1,2-*Bis*(2-aminophenoxy)ethane-*N*,*N*,*N*′,*N*′-tetraacetic acid tetrakis (acetoxymethyl ester); BK channel, large conductance Ca^{2+}-activated K^+ channel; CICR, calcium-induced calcium release; CPA, Cyclopiazonic acid; CTX, Charybdotoxin; $[Ca^{2+}]_c$, cytosolic concentration of free calcium ions; DMSO, dimethyl sulphoxide; dV/dt_L, leading slope; dV/dt_T, trailing slope; IbTX, Iberiotoxin; $[K^+]_0$, extracellular concentration of potassium ions; SK channel, small conductance Ca^{2+}-activated K^+ channel; VK channel, voltage-sensitive K^+ channels

Introduction

Bladder smooth muscle strips develop spontaneous phasic contractions (Fujii *et al*., 1990; Herrera *et al*., 2000). Underlying these contractions are thought to be spontaneous action potentials and corresponding calcium transients (Hashitani *et al*., 2001). Spontaneous action potentials result from the opening of L-type calcium channels (Mostwin, 1986), and Ca^{2+} entry through these channels would be amplified by calcium-induced calcium release (CICR) from intracellular stores, to contract bladder smooth muscles (Ganitkevich & Isenberg, 1992; Imaizumi *et al*., 1998; Hashitani *et al*., 2001). In other smooth muscles, spontaneous electrical activity, for example, slow waves and spontaneous transient depolarizations, persist in the presence of dihydropyridine blockers for L-type Ca^{2+} channels, and their generation is associated with Ca^{2+} release from intracellular stores (Van Helden, 1993; Hashitani *et al*., 1996; Ward *et al*., 2000). In contrast, spontaneous action potentials in detrusor smooth muscle were abolished by dihydropyridines. Furthermore, both spontaneous action potentials and associated Ca^{2+} transients persist during prolonged exposure to either cyclopiazonic acid (CPA)

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Advance online publication: 11 August 2003

or ryanodine, indicating that Ca^{2+} release from internal stores is not a fundamental process for the initiation of spontaneous action potentials in detrusor smooth muscles (Hashitani *et al*., 2001).

Although Ca^{2+} release from internal stores does not contribute to the action potential initiation, Ca^{2+} influx and subsequent CICR from it may cause a 'negative feedback' on L-type calcium channels by increasing cytosolic calcium concentration ($[Ca^{2+}]_c$), particularly in the subplasmalemma area (Van Breemen *et al*., 1995). Increases in subplasmalemmal Ca^{2+} will open Ca^{2+}-activated K^+ channels to repolarize the action potentials, and thus lead to the closure of L-type Ca^{2+} channels (Heppner *et al*., 1997; Imaizumi *et al*., 1998; Ohi *et al*., 2001). Consistent with this, enhanced spontaneous contractile activity in the presence of Ca^{2+}-activated K^+ channel blockers, that is, iberiotoxin (IbTX) and apamin, has been reported (Heppner *et al*., 2000). Increases in $[Ca^{2+}]_c$ beneath the plasma membrane may also inhibit the calcium influx through L-type Ca^{2+} channels by Ca^{2+}-mediated inactivation of these channels (Schneider *et al*., 1991; Nakayama & Brading, 1993; Yoshino *et al*., 1995).

Extensive studies using isolated bladder smooth muscle cells have advanced our understanding of ion channels and Ca^{2+}-mediated regulation of these channels in these cells. However, these studies were mostly carried out using cells that did not generate spontaneous action potentials (Klökner & Isenberg, 1985; Schneider *et al*., 1992), and were often performed at room temperature (Nakayama & Brading, 1993; Yoshino *et al*., 1995; Imaizumi *et al*., 1998). Furthermore, c-kit-positive cells that resemble pace-making cells in the gastrointestinal tract have been found in guinea-pig bladders (McCloskey & Gurney, 2002). If such cells are involved in controlling activity in the bladder smooth muscle, depolarizations induced in isolated detrusor smooth muscle cells may not reproduce spontaneous action potentials. The spontaneous action potentials in bladder strips have been mostly studied in preparations where contractions have been diminished by a high extracellular osmolarity (Mostwin, 1986; Fujii *et al*., 1990; Heppner *et al*., 1997). In this condition, Ca^{2+} homeostasis is likely to be different from that in physiological conditions (Proctor & Fry, 1999). Thus, only limited information is available about the regulatory mechanisms for spontaneous excitation in intact bladder smooth muscle so far (Hashitani *et al*., 2001). We now need to re-evaluate the regulation of action potentials in bladder smooth muscles under normal conditions, particularly focusing on ion channels in the plasma membrane, to fill the gap between single cell studies and contractile studies.

In the present paper, the effects of membrane polarizations induced by intracellular current injection and alternations in the temperature on the frequency of action potentials were examined. In order to elucidate the role of K^+ channels in action potential regulation, we examined the effects of blockers for Ca^{2+}-activated K^+ channels (charybdotoxin (CTX), Iberiotoxin (IbTX) and apamin H-H and for voltage-dependent K^+ channels 4-aminopyrideine (4-AP) on spontaneous action potentials. The role of intracellular Ca^{2+} stores in action potential regulation was also assessed by applying CPA, ryanodine and (BAPTA-AM) 1,2-*Bis*(2-aminophenoxy)ethane-*N*,*N*,*N*′,*N*′-tetraacetic acid tetrakis (acetoxymethl ester). To understand the link between spontaneous action potentials and contractile activity, the effects of K^+ channel blockers, intracellular calcium modulators and lowering the temperature on spontaneous phasic contractions were examined.

Methods

General

The procedures described have been approved by the animal experimentation ethics committee at the University of Oxford. Male guinea-pigs, weighing 200–400 g, were killed by a blow to the head followed by a cervical dislocation. The urinary bladder was removed and its ventral wall was opened longitudinally from the top of the dome to the bladder neck. The mucosal layer, connective tissues and several smooth muscle layers were then removed leaving an underlying single layer of smooth muscle bundles attached to the serosal layer. For microelectrode recordings, a serosal sheet that contained a single bundle of smooth muscle 2–3 mm long and 0.3–0.7 mm wide was then prepared as described previously (Hashitani *et al*., 2001). For muscle tension recordings, a serosal sheet that contained a slightly larger muscle bundle (3–5 mm long and 0.3–1 mm wide) was prepared.

Microelectrode recordings

Preparations were pinned out on a Sylgard plate (silicone elastomer, Dow Corning Corporation, Midland, MI, U.S.A.) at the bottom of a recording chamber (volume, approximately 1 ml), which was mounted on a stage of an inverted microscope. The preparations were superfused with warmed (37°C) physiological saline at a constant flow rate (2 ml min^{-1}). Individual bladder smooth muscle cells were impaled with glass capillary microelectrodes, filled with 0.5 M KCl (tip resistance, 120–250 MΩ). Membrane potential changes were recorded using a high input impedance amplifier (Axoclamp-2A, Axon Instruments, Inc., Foster City, CA, U.S.A.), and displayed on a cathode-ray oscilloscope (Data SYS 740, Gould Nicolet Technologies, Ilford, Essex, U.K.). After low-pass filtering (cutoff frequency, 1 kHz), membrane potential changes were digitized using PowerLab/4SP (ADInstruments Ltd., Grove House, Hastings, U.K.) and stored on a personal computer for later analysis.

In some experiments, following a neutralization of the tip resistance, either outward or inward current was injected into cells through the recording electrode and resultant voltage changes were recorded.

Isometric tension recordings

For isometric tension recording, one end of the preparation was pinned out on a Sylgard plate at the bottom of the recording chamber, and a thread was tied around the other end. The thread was attached to an isometric force transducer, which was connected to a bridge amplifier (ADInstruments Ltd., Grove House, Hastings, U.K.). Isometric tension changes were digitized using PowerLab/4SP and stored on a personal computer for later analysis. A tension of approximately 1 mN was applied to preparations which were then left to equilibrate for 60–90 min until spontaneous phasic contractions were generated that were stable in both amplitude and frequency.

Solution

The composition of physiological saline was (in mM): NaCl, 120; KCl, 5.9;. $MgCl_2$, 1.2; $CaCl_2$, 2.5; $NaHCO_3$, 15.5; NaH_2PO_4, 1.2 and glucose, 11.5. The solution was aerated with 95% O_2 and 5% CO_2, and solution pH was maintained at 7.2–7.3. High potassium containing solution was prepared by equimolar replacement of NaCl with KCl.

Drugs used were 4-AP (from ICN Biomedicals Ltd., Aurora, OH, U.S.A.), BAPTA-AM (from Calbiochem-Novabiochem Ltd., San Diego, CA, U.S.A.), α,β-methylene-ATP, apamin, atropine sulphate, CTX, IbTX, nifedipine, phentolamine mesylate and ryanodine (from Sigma, St Louis, MO, U.S.A.), propranolol hydrochloride (from RBI, Natrick, MA, U.S.A.), tetrodotoxin (from Alomone Lab., Jerusalem, Israel) and CPA (from Tocris Cookson Ltd., Bristol, U.K.). 4-AP, α,β-methylene-ATP, apamin, atropine, CTX, IbTX, phentolamine, propranorol and tetrodotoxin were dissolved in distilled water. Nifedipine was dissolved in 100% ethanol, and BAPTA-AM, CPA and ryanodine were dissolved in dimethyl sulphoxide (DMSO). The final concentration of these solvents in the physiological saline did not exceed 1 : 1000.

Calculations and statistic

Measured values were expressed as mean ± s.d. Statistical significance was tested using paired *t*-test, and probabilities of less than 5% different from the control were considered significant.

The following parameters of action potentials were measured: peak amplitude, measured as the value from the resting membrane potential to the action potential peak, which was defined as an average of 0.1 ms on either side of the maximum point; leading dV/dt (dV/dt_L), measured as the slope between 20 and 80% of the peak amplitude of the events on the raising phase; half-width, measured as the time between 50% peak amplitude on the rising and falling phases; and trailing dV/dt (dV/dt_T), measured as the slope between 20 and 80% of the peak amplitude of the events on the falling phase. The amplitude of after-hyperpolarizations (AHPs) was measured as the value from the resting membrane potential to the peak of the AHPs and the time constant of the decay of AHPs was also measured.

For isometric tension changes, the following parameters were measured: peak amplitude, measured as the value from the basal tension level to the peak of phasic contractions, which was defined as an average of 2 ms on either side of the maximum point; duration, measured as the time between 50% peak amplitude on the rising and falling phases; and frequency, which was defined as an average of 5 min recordings.

RESULTS

General observations

In all the preparations examined, bladder smooth muscle cells exhibited spontaneous action potentials and had resting membrane potentials, determined at the most negative potential between each action potential, which ranged between −54 and −38 mV (mean −42.9 ± 3.1 mV, $n=65$). In 53 preparations, action potentials were generated individually, and had a frequency ranging between 7 and 108 min^{-1} (mean 33.5 ± 20.1 min^{-1}). The remaining 12 preparations generated bursts of action potentials, which occurred with a frequency of 1.2 to 4.8 min^{-1} (3.2 ± 1.7 min^{-1}) with each burst consisting of four to 20 action potentials (8.4 ± 4.5 action potentials) lasting for 2 to 9 s (5.3 ± 2.2 s).

Each action potential consisted of an initial depolarizing phase and a subsequent regenerative depolarization. The regenerative depolarization was followed by a rapid repolarizing phase that caused an AHP. Action potentials had peak amplitudes ranging between 43 and 62.3 mV (mean 52.3 ± 5.1 mV), leading dV/dt (dV/dt_L) ranging between 1.3 and 5.7 mV $m s^{-1}$ (mean 2.9 ± 0.9 mV $m s^{-1}$), half-widths ranging between 4.3 and 8.9 ms (mean 6.2 ± 1.3 ms) and trailing dV/dt (dV/dt_T) raging between −7.1 and −23.1 mV $m s^{-1}$ (mean −15.1 ± 2.6 mV $m s^{-1}$). Amplitudes of AHPs ranged between 5.2 and 19.8 mV (mean 12.1 ± 2.7 mV). AHPs were followed by a quiescent period, which preceded the next action potential.

Spontaneous action potentials were abolished by nifedipine (1 μM, $n=3$; 10 μM, $n=4$, Figure 1A), indicating that they result from the opening of L-type calcium channels.

Effects of alternations in the membrane potential on spontaneous action potentials

To examine the effect of membrane polarization on spontaneous action potentials, either outward or inward currents were injected through the recording electrode in six preparations. Outward currents caused depolarizations and increased the action potential frequency (Figure 1Ba–c). The frequency of action potentials depended on the degree of membrane polarization, and some 20 mV of depolarization from the resting membrane potential increased the frequency to about 350 min^{-1}. During large depolarizations, the action potential peak was suppressed (Figure 1Ba,b). In contrast, inward current caused hyperpolarization and either reduced the frequency of spontaneous action potentials or prevented their generation (Figure 1Bd). During small hyperpolarizations, the amplitude of the action potentials was increased.

To further investigate the effect of membrane depolarizations on action potentials, the effects of altered extracellular potassium concentration ($[K^+]_0$) were examined. Increasing $[K^+]_0$ from 5.9 to 29.5 mM depolarized the membrane to −22.5 ± 1.8 mV ($n=6$), and action potentials occurred with a frequency of 77.3 ± 18.1 min^{-1} and had an amplitude of 19.8 ± 2.8 mV. Further increases in $[K^+]_0$ (41.3 mM) depolarized the membrane to around −12 mV (mean −11.6 ± 0.9, $n=5$), and almost completely suppressed action potentials.

Effects of lowering the temperature on spontaneous action potentials

The effect of lowering the temperature on spontaneous action potentials was studied in five preparations. Lowering the temperature from 36 to 25°C caused small hyperpolarizations and reduced action potential frequency (37.4 ± 8.5 min^{-1} in 36°C; 6.8 ± 2.2 min^{-1} 25°C, $P<0.05$, Figure 2Ab). In 25°C, action potentials had an increased amplitude (55.8 ± 3.9 mV in 36°C; 64.9 ± 4.1 mV in 25°C, $P<0.05$), decreased dV/dt_L (5.3 ± 1.7 mV ms^{-1} in 36°C; 2.9 ± 0.8 mV $m s^{-1}$ in 25°C,

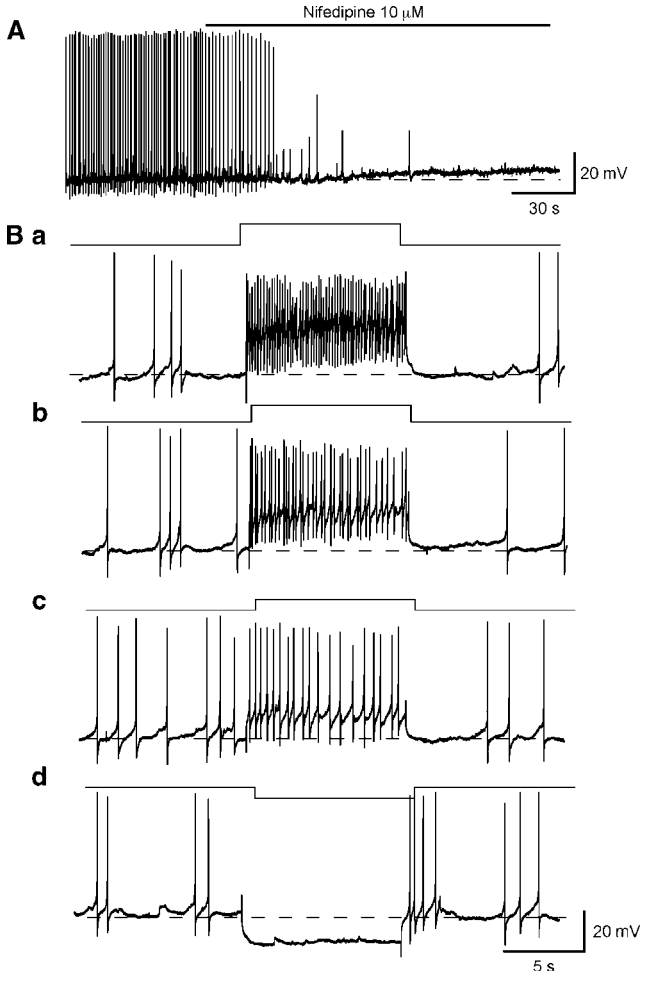

Figure 1 Effects of membrane polarization and nifedipine on spontaneous action potentials recorded from guinea-pig bladder smooth muscle. In a bladder smooth muscle preparation, nifedipine (10 μM) abolished action potentials and produced a small depolarization of the membrane (A). In another preparation, spontaneous action potentials were generated with a frequency of about 40 min^{-1} (B). Outward current injected through the recording electrode depolarized the membrane and increased action potential frequency (Ba–c). During the depolarization, the amplitude of action potentials was reduced. In the same preparation, inward current with an amplitude of 0.1 nA hyperpolarized the membrane by about 10 mV and prevented the generation of action potentials (Bd). Resting membrane potential was −46 mV in (A) and −42 mV in (B).

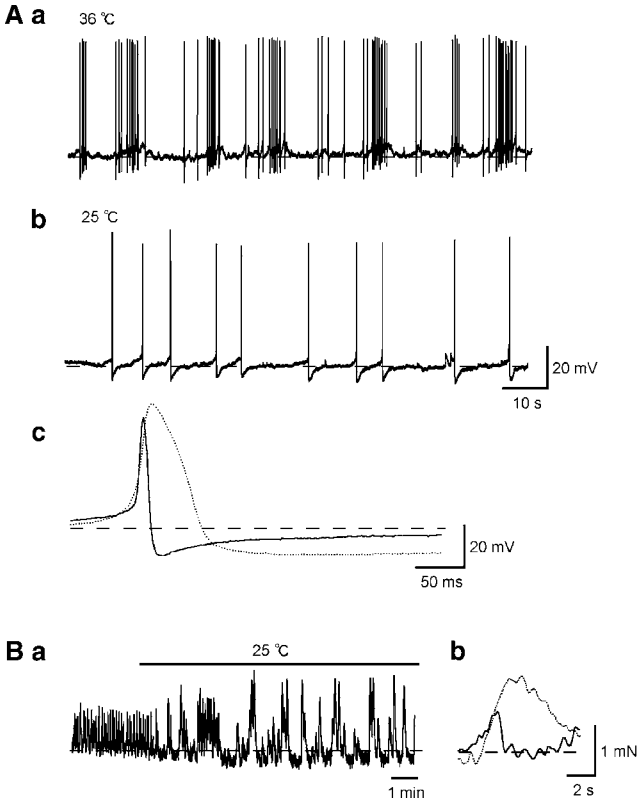

Figure 2 Effect of lowering the temperature on spontaneous electrical and mechanical activity recorded from guinea-pig bladder smooth muscle. In control condition (36°C), this preparation generated bursts of action potentials with a frequency of 50 min^{-1} (Aa). In low temperature (25°C), action potentials occurred individually with a frequency of 5 min^{-1} (Ab). On a fast time scale (Ac), each action potential in control condition had an amplitude of about 50 mV, lasted for some 10 ms, and was followed by an AHP (*full line*). Each action potential at 25°C had an increased amplitude of about 60 mV, lasted for some 40 ms, and was followed by a prolonged AHP (*dotted line*). Note that the recovery of AHPs is much slower than that in control condition. In another preparation, lowering the temperature from 36 to 25°C reduced the frequency of spontaneous contractions and increased their amplitude (Ba). On a fast time scale, spontaneous contractions in 25°C (*dotted line*) had larger amplitude and duration than those in 36°C (*full line*). Resting membrane potential was −46 mV in (Aa) and −48 mV in Ab.

$P<0.05$), decreased dV/dt_T (-14.6 ± 1.6 mV ms^{-1} in 36°C; -3.2 ± 1.1 mV $m s^{-1}$ in 25°C, $P<0.05$) and increased half-width (5.7 ± 0.6 ms in 36°C; 22.6 ± 2.8 ms in 25°C, $P<0.05$, Figure 2B). The amplitude of AHPs was also reduced (12.2 ± 2.5 mV in 36°C; 7.8 ± 2.5 mV in 25°C, $P<0.05$) and the time constant of decay of the AHPs was greatly increased (80.2 ± 25.4 ms in 36°C; 815.8 ± 309.2 ms in 25°C, $P<0.05$, Figure 2B).

The effects of lowering the temperature on spontaneous phasic contractions were examined in four preparations. Lowering the temperature from 36 to 25°C increased the amplitude of spontaneous contractions (0.82 ± 0.17 mN in 36°C, 1.2 ± 0.23 mN in 25°C, $P<0.05$, Figure 2Ba,b) and their duration (1.1 ± 0.26 s in 36°C, 3.3 ± 0.88 s in 25°C, $P<0.05$, Figure 2Bb). Lowering the temperature also reduced their frequency (8.8 ± 3.7 min^{-1} in 36°C, 2.9 ± 1.5 min^{-1} in 25°C, $P<0.05$) and the resting tension level (Figure 2Ba).

Effects of K^+ channels blockers on spontaneous action potentials

Since Ca^{2+}-activated K^+ channels are thought to contribute to the repolarization of action potentials and AHPs in bladder smooth muscles, the effects of Ca^{2+}-activated K^+ channel blockers were examined.

CTX (50 nM), a blocker of large conductance Ca^{2+}-activated K^+ channels (BK) slightly depolarized the membrane and increased action potential frequency for the initial 5–10 min (Figure 3A). During a prolonged application of CTX, the membrane potential gradually repolarized and became slightly more negative than its original values after some 15–20 min. The action potential frequency also decreased and eventually became lower than the control value (Figure 3Bb). CTX

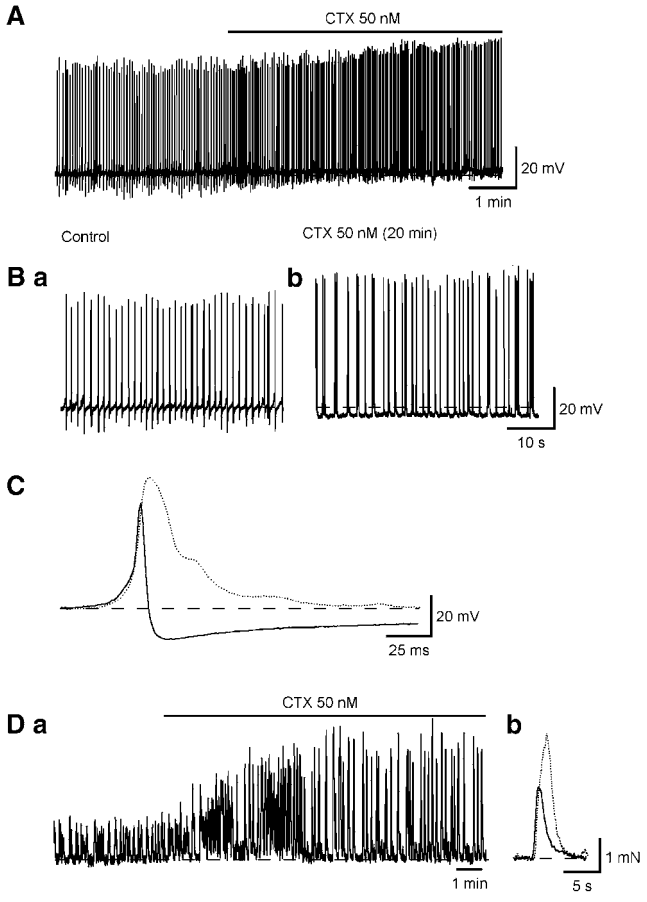

Figure 3 Effects of CTX on spontaneous electrical and mechanical activity recorded from guinea-pig bladder smooth muscle. CTX (50 nM) initially increased the frequency and amplitude of action potentials, and suppressed AHPs (A). In control condition, action potentials occurred with a frequency of about 40 min^{-1} (Ba). After some 20 min exposure to CTX, the amplitude of action potentials was increased and action potentials frequency was reduced to some 30 min^{-1} (Bb). On a fast time scale (C), each control action potential had an amplitude of about 50 mV, lasted for 10 ms and was followed by an AHP (*full line*). Each action potential in CTX had an amplitude of about 70 mV, lasted for some 40 ms, and the AHP was abolished (*dotted line*). In another preparation, CTX increased the amplitude of spontaneous phasic contractions and reduced their frequency (Da), shown on a fast time scale in (Db) (*full line* in control, *dotted line* in CTX, Db). (A–C) were recorded from the same cell. Resting membrane potential was −41 mV.

increased the amplitude by about 30%, the half-width by about 330% and dV/dt_L by about 40%, and decreased dV/dt_T to about 13% of control values (Figure 3C, Table 1). CTX also abolished AHPs ($n=10$; Figure 3Bb, C).

The effects of CTX (50 nM) on spontaneous phasic contractions were examined in five preparations. CTX increased the amplitude of spontaneous contractions (1.9 ± 1.5 mN in control, 3.7 ± 1.5 mN in CTX, $P<0.05$, Figure 3Da,b) and the duration (2.9 ± 1.3 s in control, 4.2 ± 2.4 s in CTX, $P<0.05$, Figure 3Db). CTX initially increased the frequency of phasic contractions but then reduced it below the control value (5.3 ± 4.4 min^{-1} in control, 3.4 ± 3 min^{-1} in CTX, $P<0.05$, Figure 3D).

IbTX (0.1 μM), a highly selective blocker for BK channels had very similar effects on spontaneous action potentials to those of CTX. IbTX initially increased the frequency of action potentials for 5–10 min, but then reduced it below the control value with a small hyperpolarization of the membrane (Figure 4Aa,b). In the presence of IbTX for some 20 min, action potentials had an increased amplitude and duration, and AHPs were abolished (Figure 4Ab and B, Table 1, $n=4$). During AHPs, the membrane potential changes induced by intracellular current injection had smaller amplitude and time constant than those in following slow depolarizing phase (Figure 4B), suggesting that AHPs result from increases in ionic conductance.

The effects of IbTX (0.1 μM) on spontaneous phasic contractions were examined in three preparations. Again, IbTX had very similar effects on spontaneous contractions to those of CTX. IbTX increased the amplitude of spontaneous phasic contractions (1.5 ± 0.2 mN in control, 4.0 ± 1.1 mN in IbTX, $P<0.05$, Figure 4Ca,b) and the duration (2.0 ± 0.5 s in control, 3.0 ± 0.2 s in IbTX, $P<0.05$, Figure 4Cb). IbTX initially increased the frequency of phasic contractions but then reduced it below the control value (7.3 ± 4.6 min^{-1} in control, 3.8 ± 3.1 min^{-1} in IbTX, $P<0.05$, Figure 4 Ca).

Apamin (0.1 μM), a blocker of small conductance Ca^{2+}-activated K^+ channels (SK), caused a small depolarization of the membrane (mean 3.2 ± 0.8 mV, $n=10$) and converted individual action potentials into bursts in eight preparations (Figure 5A and Bb). Apamin did not change either the amplitude or the time course of action potentials (Figure 5C, Table 1). Unlike previous reports carried out in hyperosmolar solutions (Fujii *et al*., 1990), apamin failed to reduce the amplitude of AHPs (12.2 ± 1.8 mV in control; 12.9 ± 1.4 mV in apamin, $n=10$, $P>0.05$; Figure 5C). A higher concentration of apamin (1 μM) caused small sustained depolarization during bursts of action potentials but failed to prevent AHPs ($n=3$).

The effects of apamin (0.1 μM) on spontaneous contractions were examined in four preparations. Apamin increased the amplitude of spontaneous phasic contractions (1.3 ± 0.5 mN in control, 2.8 ± 0.9 mN in apamin, $P<0.05$, Figure 5Da,b) and the duration (2.0 ± 0.8 s in control, 3.8 ± 0.5 s in apamin, $P<0.05$, Figure 5Db). Apamin also reduced the frequency of spontaneous contractions (5.8 ± 1.5 min^{-1} in control, 2.4 ± 0.7 min^{-1} in apamin, $P<0.05$, Figure 5Da).

Finally, the effect of 4-AP, a blocker for voltage-dependent K^+ (KV) channels, on spontaneous action potentials was examined. 4-AP (1 mM) caused small depolarization and increased the frequency of action potentials (Figure 6A and B). 4-AP did not affect either action potential parameters (Figure 6C, Table 1) or AHPs (11.1 ± 2.2 mV in control, 11.5 ± 3.2 mV in 4-AP, $P>0.05$, $n=5$).

The effects of 4-AP (1 mM) on spontaneous contractions were examined in five preparations. In three preparations, 4-AP increased the frequency of spontaneous phasic contractions by about 400% (Figure 6D). During the application of 4-AP, the basal tension level was increased and the amplitude of phasic contractions was reduced (Figure 7D). In the remaining two preparations, 4-AP caused sustained contractions with an amplitude of about 2 mN, which were interrupted by transient relaxations.

To assess the possible effects of K^+ channel blockers on neurotransmitter release, a series of experiments were carried out in the presence of a cocktail containing blockers for the neurotransmitter (atropine, 1 μM, muscarinic antagonist; α,β-methylene-ATP, 10 μM, P_{2X} desensitizer; phentolamine, 1 μM, α-antagonist; propranolol, 1 μM, β-antagonist; tetrodotoxin,

Table 1 Effects of K^+ channel blcockers and modulators of intracellular Ca^{2+} on the parameters of spontaneous action potentials in the guinea-pig bladder

Compound (M)	*Amplitude* (mV)	dV/dt_L (mV ms^{-1})	dV/dt_T (mV ms^{-1})	*50% width* (ms)
Control ($n=10$)	52.4±1.6	2.9±0.2	−16.0±0.8	6.3±0.5
CTX (50 nM)	67.1±7.6*	3.3±0.9	−1.7±1.1*	27.2±5.9*
Control ($n=4$)	53.0±2.5	3.2±0.4	−14.9±0.9	6.6±0.7
IbTX (0.1 μM)	67.9±6.2*	2.9±0.7	−0.7±0.3*	85.2±24.6*
Control ($n=10$)	50.8±2.0	2.8±0.3	−15.4±0.7	6.6±0.4
Apamin (0.1 μM)	49.8±1.7	2.9±0.3	−14.7±2	6.8±0.9
Control ($n=5$)	50.9±1.9	3.0±0.2	−14.4±2.2	6.6±0.4
4−AP (1 mM)	52.3±1.9	2.9±0.4	−14.2±1.1	6.7±0.7
Control ($n=6$)	53.6±1.5	3.1±0.5	−16.2±0.7	6.3±0.8
CPA (10 μM)	60.9±2.8*	3.5±0.8	−6.2±2.8*	11.7±3.6*
Control ($n=4$)	52.1±3.4	3.1±0.4	−15.6±1.1	5.5±0.8
Ryanodine (50 μM)	60.0±3.1*	2.9±0.2	−6.8±1.6*	9.0±1.5*
Control ($n=7$)	52.1±2.1	3.0±0.5	−14.5±1.9	6.9±0.9
BAPTA-AM (50 μM)	80.8±9.2*	4.5±1.4*	−0.08±0.06*	975.3±353.4*

Data shown are mean±s.d.
*Significantly different from control values ($P<0.05$).

1 μM, voltage-dependent Na^+ channel blocker). Spontaneous action potentials persisted in the presence of the drug cocktail, indicating that the generation of spontaneous action potentials is myogenic origin. In the presence of the drug cocktail, bursts of action potentials, which were similar to those induced by apamin, were generated ($n=5$). Applications of each neurotransmitter blocker indicated that phentolamine (1 μM) converted individual action potentials into bursts ($n=3$). We did not investigate this further.

In the presence of the drug cocktail, effects of each K^+ channel blocker on spontaneous action potentials were very similar to those in control solutions. CTX (50 nM) initially increased the frequency of action potentials and then reduced their frequency below control levels with membrane hyperpolarizations. In the presence of CTX for some 20 min, action potential had an increased amplitude and often showed sustained depolarizations that lasted about 10 s, and lacked AHPs ($n=3$). Apamin caused small depolarizations and increased the duration of action potential burst but did not inhibit AHPs ($n=3$). 4-AP increased the frequency of action potentials without obviously changing either action potential shapes or AHPs ($n=3$). These results suggested that K^+ channel blockers modulated spontaneous action potentials by blocking targeted K^+ channels on smooth muscle membrane rather than by affecting neurotransmitter release.

Effects of CPA on spontaneous action potentials

To clarify whether or not CICR from intracellular stores contributes to the opening of BK channels, the effects on action potentials of CPA, a blocker for the sarcoplasmic reticulum Ca^{2+} ATPase were studied. CPA (10 μM) initially depolarized the membrane and increased action potential frequency for 5–10 min, although in preparations that generated spontaneous action potentials with a relatively high frequency, its initial effect on action potential frequency was not obvious (Figure 7A). During a prolonged application of CPA (some 20–30 min), the membrane potential repolarized and became slightly negative to the control level. The action potential frequency also became lower than the control value (Figure 7Ba,b). CPA increased the amplitude by 20% and the half-width by 80% and decreased dV/dt_T to about 53% of control values without changing dV/dt_L (Figure 7C, Table 1). CPA almost completely suppressed AHPs ($n=6$; Figure 7Bb,C).

To assess the possible effects of the disruption of internal calcium stores on neurotransmitter release, effects of CPA on spontaneous action potentials were examined in the presence of the drug cocktail used above. In the presence of the drug cocktail, CPA had similar effects to those in the control solution. CPA initially caused small depolarizations and increased the action potential frequency. After a prolonged application of CPA (20–30 min), CPA increased the duration of action potential bursts and suppressed AHPs with membrane hyperpolarizations ($n=3$).

The effects of CPA on spontaneous phasic contractions were examined in five preparations. CPA initially increased the frequency of spontaneous contractions and often induced prolonged or sustained contractions (Figure 7Da). After a prolonged exposure to CPA (some 20 min), spontaneous phasic contractions had an increased amplitude (1.7±0.9 mN in control, 2.4±1.5 mN in CPA, $P<0.05$, Figure 7Da,b), an increased duration (3.0±0.9 s in control, 6.3±2.9 s in CPA, $P<0.05$, Figure 7Db) and a reduced frequency (4.0±2.7 min^{-1} in control, 1.1±0.5 min^{-1} in CPA, $P<0.05$, Figure 7Da).

Effects of ryanodine on spontaneous action potentials

To investigate the role of ryanodine receptors on the intracellular calcium store membrane in the activation of BK channels, the effects of ryanodine on action potentials were examined.

Ryanodine (50 μM) initially depolarized the membrane and increased action potential frequency for 5–10 min (Figure 8A).

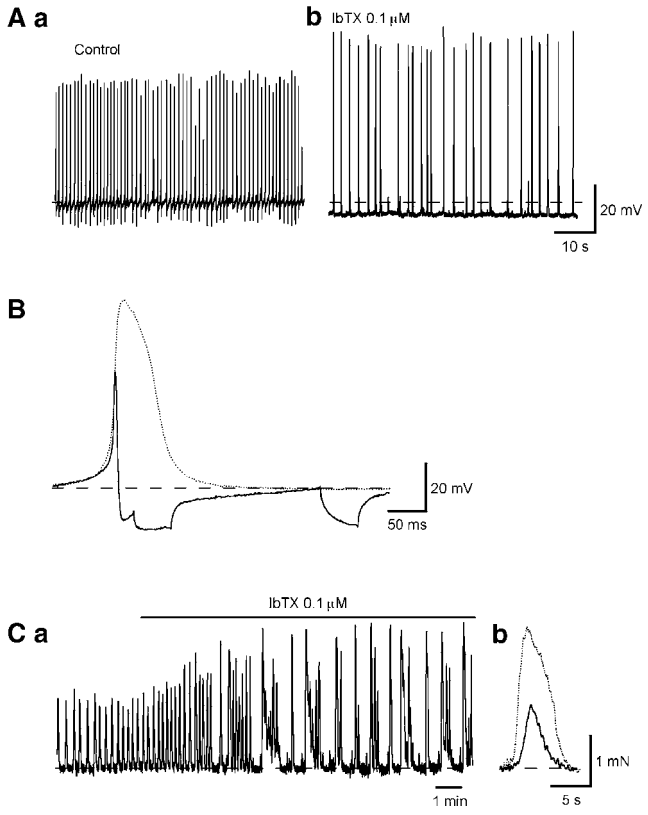

Figure 4 Effects of IbTX on spontaneous electrical and mechanical activity recorded from guinea-pig bladder smooth muscle. In control conditions, action potentials occurred with a frequency of about 60 min^{-1} (Aa). After some 20 min exposure to IbTX (0.1 μM), the action potential amplitude was increased and the frequency was reduced to some 25 min^{-1} (Ab). On a fast time scale (B), each control action potential had an amplitude of about 50 mV, lasted for 10 ms and was followed by an AHP (*full line*). Note that membrane potential changes induced by intracellular current injection had a smaller amplitude and faster time constant during the AHP. Each action potential in IbTX had an amplitude of about 75 mV, lasted for some 50 ms, and an AHP was abolished (*dotted line*). In another preparation, IbTX increased the amplitude of spontaneous contractions and reduced their frequency (Ca), shown on a fast time scale in Cb (*full line* in control, *dotted line* in IbTX, Cb). Traces in (A and B) were recorded from the same cell. Resting membrane potential was −44 mV.

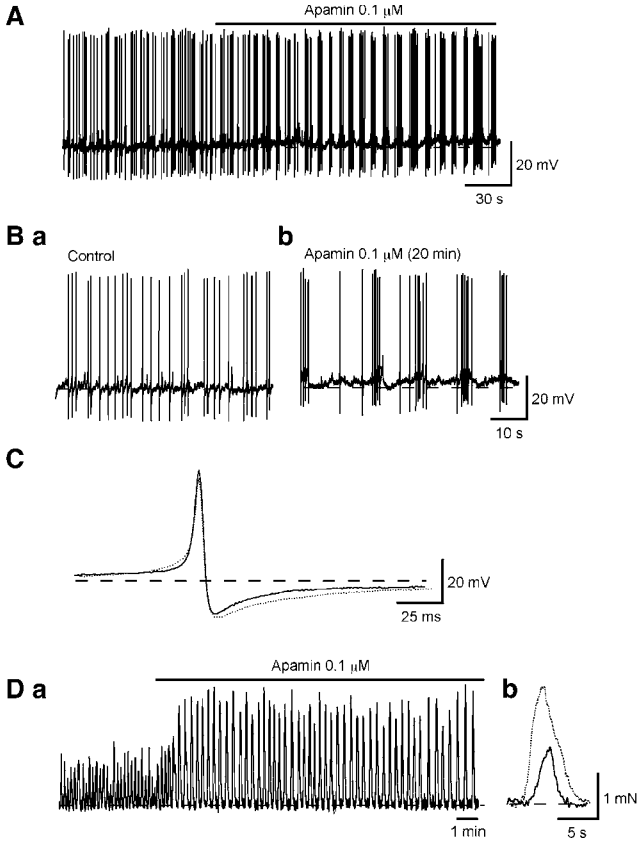

Figure 5 Effects of apamin on spontaneous electrical and mechanical activity recorded from guinea-pig bladder smooth muscle. Apamin (0.1 μM) caused a small depolarization and converted continuous generation of action potentials into bursts (A). In control conditions, action potentials had an amplitude of about 40 mV and occurred with a frequency of 30 min^{-1} (Ba). After some 20 min exposure to apamin, the action potential amplitude was unchanged but bursts of action potentials were generated (Bb). On a fast time scale (C), the shape of action potentials in apamin (*dotted line*) were almost identical to that of control action potentials (*full line*). In a different preparation, apamin increased the amplitude and reduced the frequency of the phasic contractions. (D), shown on a fast time in (Db) (*full line* in control, *dotted line* in apamin). Resting membrane potential was −39 mV in (A–C).

During a prolonged application of ryanodine (for some 30 min), the membrane potential repolarized and became slightly more negative than its original level. The action potential frequency also became lower than in control conditions (Figure 8Bb). Ryanodine increased the amplitude by 16% and the half-width by 60% and decreased dV/dt_T to some 47% of control values without changing dV/dt_L (Figure 8C, Table 1). Ryanodine almost completely suppressed AHPs ($n=4$; Figure 8Bb and C).

The effects of ryanodine on spontaneous contractions were examined in three preparations. Ryanodine initially increased the frequency of spontaneous contractions and caused some prolonged contractions (Figure 8Da). After a prolonged exposure to ryanodine (20–30 min), spontaneous phasic contractions had an increased amplitude (1.3 ± 0.5 mN in control, 2.8 ± 0.9 mN in ryanodine, $P<0.05$, Figure 8Da,b), an increased duration (2.0 ± 0.8 s in control, 3.8 ± 0.5 s in ryanodine, $P<0.05$, Figure 8Db) and a decreased frequency (5.8 ± 1.5 min^{-1} in control, 2.4 ± 0.7 min^{-1} in ryanodine, $P<0.05$, Figure 8Da).

Effects of BAPTA-AM on spontaneous action potentials

To further examine $[Ca^{2+}]_c$-induced inhibition of action potentials, the effects of BAPTA-AM, a chelator of intracellular calcium, on action potentials were examined. BAPTA-AM (50 μM) initially depolarized the membrane and increased action potential frequency (Figure 9A), but then gradually caused hyperpolarization and decreased the frequency (Figure 9Bb). BAPTA-AM increased the amplitude by 70%, the half-width about 140 times and dV/dt_L by 105%, and decreased dV/dt_L to 0.6 % of control values (Figure 9C, Table 1). BAPTA-AM also abolished AHPs ($n=7$; Figure 9Bb,C). During the repolarizing phase of prolonged action potentials, the amplitude and time constant of the membrane, measured by intracellular current injection, increased (Figure 9C), indicating that decreases in ionic conductance contribute to the repolarizing phase.

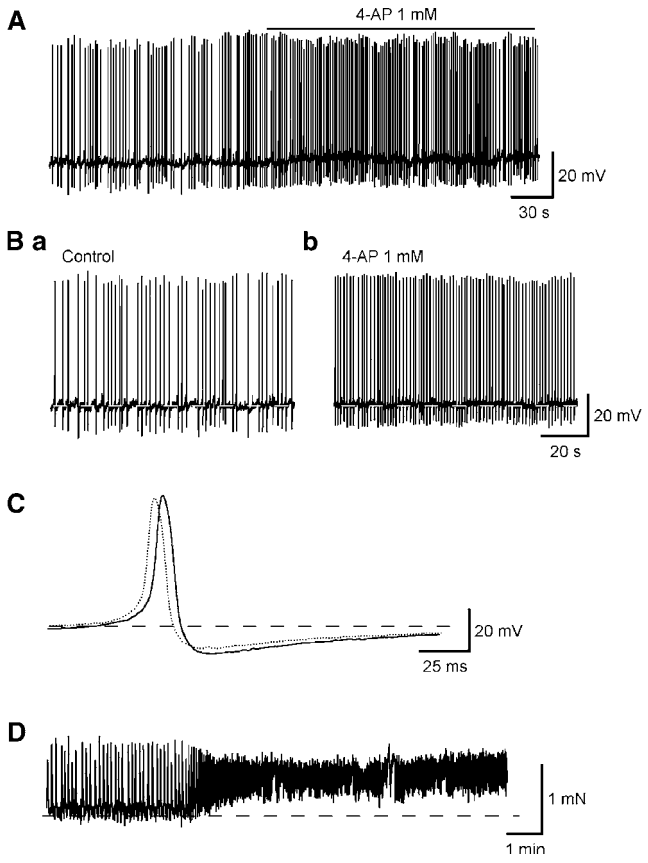

Figure 6 Effects of 4-AP on spontaneous action potentials recorded from guinea-pig bladder smooth muscle. 4-AP (1 mM) caused a small depolarization and increased action potential frequency (A). In control conditions, action potentials had an amplitude of about 50 mV and occurred with a frequency of 25 min^{-1} (Ba). After about 20 min exposure to 4-AP, the action potential frequency was increased to about 70 min^{-1} (Ca). On a fast time scale (C), the shape of action potentials in 4-AP (*dotted line*) was almost identical to that of control action potentials (*full line*). In another preparation, 4-AP (1 mM) increased the frequency of spontaneous phasic contraction and basal tension level (D). Resting membrane potential was −45 mV in (A–C).

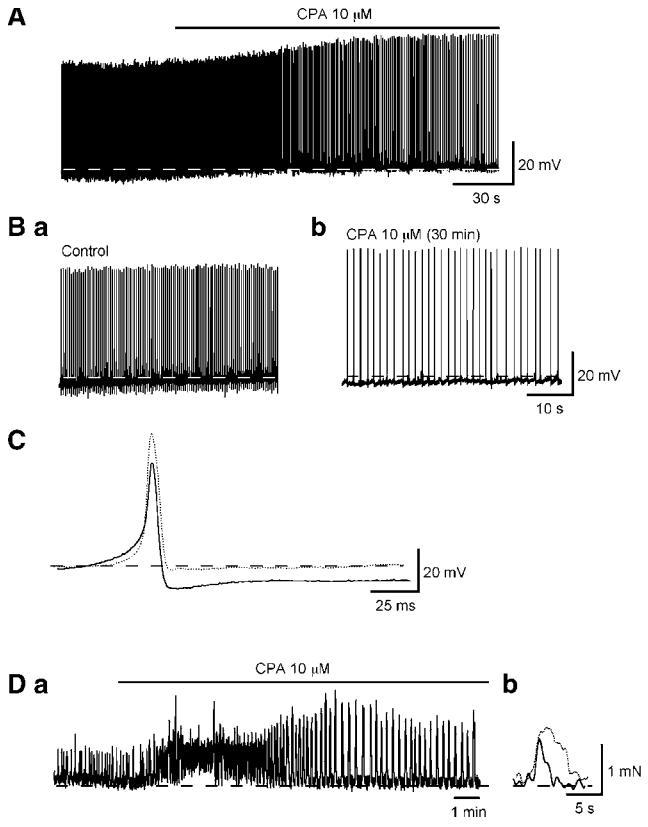

Figure 7 Effects of CPA on spontaneous electrical and mechanical activity recorded from guinea-pig bladder smooth muscle. CPA (10 μM) increased the amplitude of action potentials and gradually decreased their frequency (A). In control condition, action potentials occurred with a frequency of 80 min^{-1} (Ba). After about 30 min exposure to CPA, the action potential frequency was reduced to some 30 min^{-1} (Bb). On a fast time scale (C), each control action potential had an amplitude of about 50 mV, lasted for some 10 ms, and was followed by an AHP (*full line*). Each action potential in CPA had an increased amplitude of about 60 mV, lasted for some 15 ms, and the AHP was abolished (*dotted line*). In a different preparation, CPA initially increased the frequency of spontaneous contractions and caused a sustained contraction, later the frequency was reduced and the amplitude increased (Da), shown on a fast time scale in Db (*full line* in control, *dotted line* in CPA). All traces were recorded from the same cell. Resting membrane potential was −40 mV.

Discussion

In the present study, the ionic basis for the regulation of spontaneous action potentials in bladder smooth muscle cells of the guinea-pig was investigated using intracellular recording techniques in solutions of normal osmolarity to preserve the physiological cellular conditions. Action potentials in bladder smooth muscles result from the opening of L-type Ca^{2+} channels, and increases in $[Ca^{2+}]_c$ caused a 'negative feedback' on these channels by activating BK channels and by Ca^{2+}-mediated inactivation of L-type Ca^{2+} channels.

AHPs and the quiescent periods, which precede an action potential are important in regulating action potential frequency, which in some way determines the frequency of spontaneous contractions. In previous studies that were carried out in hyperosmolar solution to diminish muscle contractions, AHPs in bladder smooth muscles were effectively abolished by apamin, an SK channel blocker (Fujii *et al*., 1990). Furthermore, IbTX, a selective blocker for BK channels, increased the amplitude and duration of action potentials but failed to block AHPs (Heppner *et al*., 1997). In contrast, the present study demonstrated that AHPs in bladder smooth muscles were blocked by either CTX or IbTX but not apamin, suggesting that they result from the opening of BK channels but not SK channels. This discrepancy could be explained by the difference in the osmolarity of the extracellular solution. In ileal smooth muscle, AHPs recorded in normal osmotic condition were indeed inhibited by either CPA or CTX (Uyama *et al*., 1993). Unfortunately, our preliminary experiments using hyperosmotic solution were not able to reproduce previous observations (Fujii *et al*., 1990). However, the apamin we used had similar effects on contractile responses to those of the previous report (Herrera *et al*., 2000). Furthermore, apamin (0.1 μM) effectively diminished IJPs in gastric smooth muscles, which have been known to result from the opening of apamin-sensitive K^+ channels. Therefore, it is very unlikely that apamin used had become ruined, but we do not yet have any clear explanation about the discrepancy.

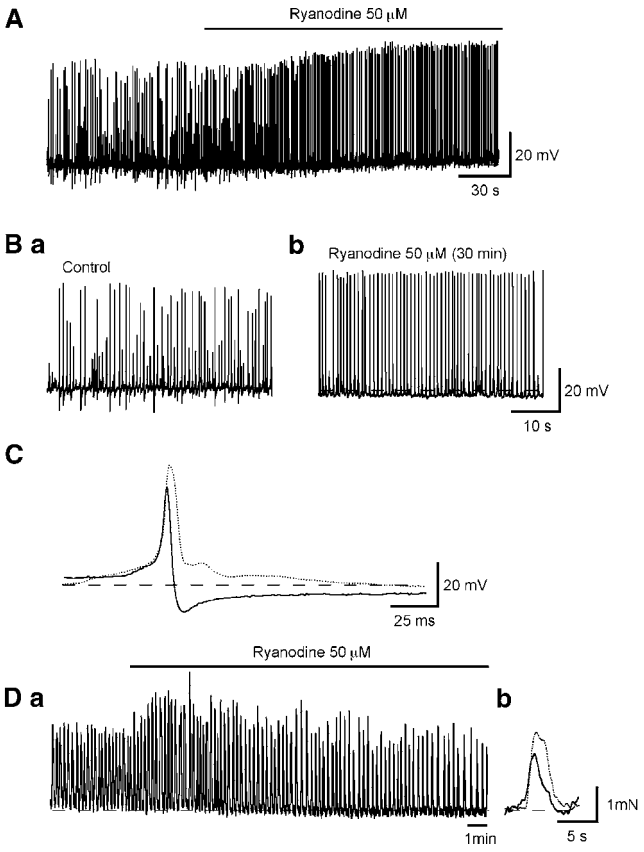

Figure 8 Effects of ryanodine on spontaneous electrical and mechanical activity recorded from guinea-pig bladder smooth muscle. Ryanodine (50 μM) initially increased the frequency and amplitude of action potentials, and inhibited AHPs (A). In control condition, action potentials occurred with a frequency of 50 min^{-1} (Ba). After some 30 min exposure to ryanodine, the action potentials frequency was reduced (Bb). On a fast time scale (C), each control action potential had an amplitude of about 45 mV, lasted for some 10 ms, and was followed by an AHP (*full line*). Each action potential in ryanodine had an amplitude of about 60 mM, lasted for some 15 ms, and the AHP was abolished (*dotted line*). In another preparation, ryanodine initially increased the amplitude and frequency of spontaneous contractions, but then reduced the frequency (Da); an example on a fast time scale is shown in (Db) (*full line* in control, *dotted line* in ryanodine). All traces were recorded from the same cell. Resting membrane potential was −39 mV.

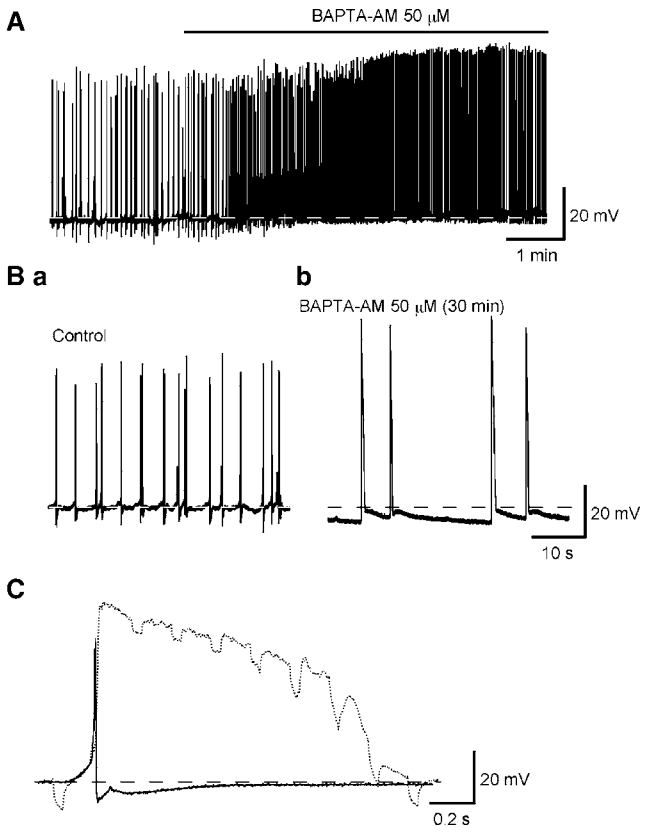

Figure 9 Effects of BAPTA-AM on spontaneous action potentials recorded from guinea-pig bladder smooth muscle. BAPTA-AM (50 μM) initially increased the frequency and amplitude of action potentials, and inhibited AHPs (A). In control condition, action potentials occurred with a frequency of 20 min^{-1} (Ba). After some 30 min exposure to BAPTA-AM, the amplitude of action potentials was greatly increased and their frequency was reduced to some 5 min^{-1} (Ca). On a fast time scale (C), each control action potential had an amplitude of about 50 mV, lasted for some 10 ms and was followed by an AHP (*full line*). Each action potential in BAPTA-AM had an amplitude of about 70 mV, lasted for some 1.2 s, and the AHP was abolished (*dotted line*). Note that the amplitude and time constant of membrane potential changes induced by intracellular current injection increased during the repolarizing phase of the action potential in BAPTA-AM. All traces were recorded from the same cell. Resting membrane potential was −41 mV.

The inhibition of BK channels by IbTX has been reported to increase the amplitude of phasic contractions and reduce their frequency in bladder smooth muscles (Herrera *et al*., 2000). Consistently, in the present study, both CTX and IbTX increased the amplitude and duration of action potentials and reduced their frequency. Furthermore, CTX and IbTX increased the amplitude and duration of spontaneous contraction and reduced their frequency in preparations that were similar to those used for intracellular recordings, suggesting that the modulation of electrical activity closely linked to changes in contractile activity.

Although the blockade of SK channels did not change either the repolarizing phase of action potentials or AHPs in normal osmolarity, apamin often converted individual action potentials into bursts. This result is consistent with a previous report in which apamin increased the amplitude and reduced the frequency of phasic contractions (Herrera *et al*., 2000). In the present study, apamin had very similar effects on spontaneous phasic contraction to those in the previous report, again indicating a close correlation between electrical and mechanical activity. Simultaneous recordings of electrical and mechanical activity in detrusor smooth muscle preparations showed that each action potential was associated with a phasic contraction (unpublished observations). In the same study, apamin converted individual action potentials into bursts, and summed phasic contractions to initiate larger contractions. Following an action potential, SK channels may undergo a more prolonged activation than BK channels and then slowly close during the quiescent period that leads to the next action potential. The blockade of SK channels indeed depolarized the membrane and shortened the interval between action potentials to induce bursts. A recent study in isolated detrusor smooth muscle cells indicated that SK channels are activated by Ca^{2+} influx through L-type Ca^{2+} channels but not Ca^{2+}

release from internal stores, and are well suited for sensing global Ca^{2+} levels (Herrera & Nelson, 2002). It has been reported that increases in global $[Ca^{2+}]_c$ have a prolonged time course compared to that of the subplasmalemmal area (Imaizumi *et al*., 1998). Therefore, SK channels may stay open until the excess calcium is cleared by the Ca^{2+} buffering systems.

Since the repolarizing phase of action potentials was suggested to be mediated by the activity of both VK channels and BK channels (Klökner & Isenberg, 1995), the blockade of VK channels was expected to decrease dV/d_{t_L} and increase the duration of action potentials. However, 4-AP increased the frequency of action potentials but did not change the action potential parameters, suggesting that VK channels contribute to the quiescent period rather than the repolarizing phase under the present experimental conditions. Since BK channels play a dominant role in the repolarizing phase, the contribution of VK channels may be relatively small; however, since the inactivation process of these channels is much slower than the activation process, they may remain open during the quiescent periods.

Calcium release from intracellular stores seems to have two 'opposite' effects on the activity of bladder smooth muscles. Several studies indicate that calcium entry through L-type channels stimulates CICR from intracellular stores to contract muscles (Ganitkevich & Isenberg, 1992; Imaizumi *et al*., 1998; Hashitani *et al*., 2001). In contrast, ryanodine has been reported to increase the frequency but not the amplitude of spontaneous phasic contractions, suggesting that calcium release from stores has a 'negative feedback' on L-type calcium channels (Herrera *et al*., 2000). In the present study, CPA and ryanodine increased the amplitude of action potentials and suppressed AHPs, again indicating that the released calcium has a 'negative feedback' effect on L-type calcium channels thought the opening of BK channels. A double immunostaining study revealed colocalization of BK channels and ryanodine receptors (Ohi *et al*., 2001), therefore, the released calcium may have a preferential access to BK channels. However, unlike results obtained from contractile studies (Herrera *et al*., 2000), in the present study, ryanodine eventually reduced action potential frequency. This discrepancy could be explained by differences in either the concentration or application time of ryanodine. Although, ryanodine (10 μM) completely inhibited the phasic components of depolarization-induced Ca^{2+} transients in isolated bladder smooth muscle cells (Ganitkevich & Isenberg, 1992), this concentration may not be sufficient for multicellular preparations. Ryanodine (10 μM) may enhance spontaneous contractions by stimulating ryanodine receptors rather than by disrupting 'negative feedback' mechanisms. In the present study, both ryanodine (50 μM) and CPA (10 μM) initially increased the frequency of spontaneous contractions and caused either prolonged or sustained contractions, but then, reduced contraction frequency.

BAPTA-AM had much stronger effects on action potentials than those induced by the blockade of BK channels, suggesting that Ca^{2+}-mediated inactivation on L-type calcium channels plays an important role in the repolarizing phase of action potentials. In the presence of BAPTA-AM, the input resistance of preparations was increased during the repolarizing phase of prolonged action potential, indicating the reduction of membrane conductance. A previous study performed in single bladder smooth muscle cells, showed that intracellular dialysis of EGTA or BAPTA diminished the suppression of calcium currents by Ca^{2+}-mediated inactivation (Schneider *et al*., 1991; Yoshino *et al*., 1995). Since bladder smooth muscle cells continued to generate action potentials at a membrane potential around -15 mV, L-type calcium channels in the bladder seem to be relatively resistant to 'voltage-dependent' inactivation, and both Ca^{2+}-mediated inactivation of L-type calcium channels and opening of BK channels play a critical role in the regulation of spontaneous action potentials (Nakayama & Brading, 1993). CPA and ryanodine suppressed AHPs but had much smaller effects on the repolarizing phase of action potentials, suggesting that Ca^{2+} influx mainly contributes to Ca^{2+}-mediated inactivation of L-type calcium channels and CICR is not required for this inactivation process as indicated in isolated bladder smooth muscle cells (Yoshino *et al*., 1995).

In the present study, BK channel blockers and modulators of intracellular calcium initially depolarized the membrane but then caused hyperpolarizations. In the presence of neurotransmitter blockers, CTX and CPA still caused the biphasic membrane potential changes, suggesting that these responses do not result from neurotransmitter release. Since BK channel blockers, CPA and ryanodine are expected to increase $[Ca^{2+}]_c$, they may activate Ca^{2+}-activated channels. Initially, Ca^{2+}-activated inward current pathways might be activated and thus the membrane depolarized. After some period, the activation of Ca^{2+}-activated K^+ channels, probably SK channels might become dominant, and thus the membrane hyperpolarized. In the case of BAPTA-AM, the reduction in $[Ca^{2+}]_c$ might close populations of Ca^{2+}-activated K^+ channels to depolarize the membrane, but further reduction in $[Ca^{2+}]_c$ might close Ca^{2+}-activated inward current pathways to cause hyperpolarizations. Unfortunately, we do not have any good evidence about what underlies the biphasic membrane potential change in either case.

The frequency of action potential was increased by membrane depolarization induced by intracellular current injections, and their generation was prevented by membrane hyperpolarization, indicating that mechanisms underlying the generation of spontaneous action potentials are highly voltage sensitive. These results are consistent with the previous study performed in hyperosmolar solutions using extracellular polarization (Mostwin, 1986).

In the present study, lowering the temperature, from 36 to 25°C substantially reduced the frequency of action potentials in detrusor smooth muscle. The frequency of slow waves recorded from gastrointestinal smooth muscle is highly sensitive to lowering the temperature but not membrane polarization, suggesting that slow waves are controlled by metabolic processes (Oba *et al*., 1975). Indeed, Ca^{2+} release from intracellular Ca^{2+} stores and subsequent mitochondrial uptake of Ca^{2+} play an important role in their generation (Ward *et al*., 2000). Therefore, in addition to voltage-dependent mechanisms, some metabolic process may regulate action potentials frequency in bladder smooth muscle preparations. Lowering the temperature prolonged the decay of AHPs and prolonged the following quiescent periods thus reducing action potential frequency. Furthermore, lowering the temperature increased both amplitude and duration of spontaneous phasic contractions. These results suggest that Ca^{2+} buffering, particularly by internal stores, was diminished

by lowering the temperature, and BK and SK channels remained open for longer periods, although the temperature sensitivity of the channel kinetics may play a role. It has been shown that increasing the temperature from 22 to 36°C increased ATP-induced inward current but reduced the amplitude of associated Ca^{2+} transients, suggesting that warming the solution stimulates Ca^{2+} sequestration and Ca^{2+} efflux (Schneider *et al*., 1991). Since many studies using isolated bladder smooth muscle cells have been carried out at room temperature, for example, 22–28°C (Nakayama & Brading, 1993; Yoshino *et al*., 1995; Imaizumi *et al*., 1998), we should take into account the diminished Ca^{2+} buffering under such conditions. Both activation and inactivation of L-type calcium channels in smooth muscle are temperature dependent (Mastuda *et al*., 1990); therefore, the slow time course of action potentials may be attributed to the altered properties of L-type channels.

In conclusion, spontaneous action potentials in the bladder smooth muscle result from the opening L-type calcium channels. During action potentials, the calcium entry opens BK channels and causes 'calcium-mediated' inhibition on L-type calcium channels to repolarize action potentials. The calcium influx also triggers CICR from intracellular stores *via* ryanodine receptors to generate AHPs. Both SK channels and voltage-dependent K^+ channels may contribute to the quiescent period leading to action potential generation and regulate their frequency. The frequency of spontaneous action potentials is highly voltage sensitive, and some metabolic process, presumably Ca^{2+} buffering by internal stores, may also regulate action potential frequency by affecting BK and SK channel activity. These regulatory mechanisms for spontaneous action potentials are closely related to the modulation of spontaneous phasic contractions, and are important in controlling the contractility of detrusor muscles.

This work was supported by grants from Action Research, Japan Society for the Promotion of Science and Uehara Memorial Foundation. We thank Wyeth Research for the gift of some of the compounds used.

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(Received November 26, 2002
Revised March 28, 2003
Accepted April 9, 2003)